

Evaluation of Anti Inflammatory and Immunomodulatory Activity of Extract of *Cucumis Sativus* in Rats

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Abstract:

The present study was designed to evaluate the anti-inflammatory and immunomodulatory potential of the ethanolic extract of *Cucumis sativus* peel (EECS) using *in vitro* and *in vivo* experimental models. The extract was obtained as a greenish-brown solid with a percentage yield of 21.5%. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, terpenoids, tannins, saponins, and phenolic compounds. Quantitative estimation demonstrated a high total phenolic content (161.05 ± 1.23 mg GAE/g) and total flavonoid content (124.26 ± 2.12 mg QE/g), indicating strong antioxidant potential. The *in vitro* anti-inflammatory activity assessed by hypotonicity-induced human red blood cell (HRBC) membrane stabilization showed concentration-dependent protection, with 72.97% inhibition of hemolysis at 1000 µg/mL, comparable to diclofenac. In the carrageenan-induced paw edema model, EECS exhibited significant anti-inflammatory activity, particularly at 400 mg/kg, reducing paw volume in both acute and repeated administration studies. Repeated administration showed greater inhibition of edema across different time intervals compared to single-dose treatment. Furthermore, cytokine analysis revealed that EECS significantly decreased serum levels of the pro-inflammatory cytokine TNF- α and increased the anti-inflammatory cytokine IL-10, especially after repeated administration. These findings confirm the immunomodulatory potential of EECS. The study concludes that EECS possesses significant anti-inflammatory activity, possibly mediated through inhibition of pro-inflammatory mediators and modulation of cytokine responses, suggesting its potential therapeutic application in inflammatory disorders

Keywords: *Cucumis sativus* peel, Ethanolic extract, Anti-inflammatory activity, TNF- α , IL-10, Phenolic content, Flavonoids

Introduction

Inflammation is a complex biological response of vascular tissues to harmful stimuli such as pathogens, damaged cells, toxic compounds, or physical injury. It functions as a protective mechanism aimed at eliminating the cause of cell injury and initiating tissue repair [1]. The inflammatory response involves activation of immune cells and release of chemical mediators including prostaglandins, leukotrienes, nitric oxide (NO), and pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α) [2]. Although essential for host defense, persistent or uncontrolled inflammation contributes to the pathogenesis of chronic diseases including rheumatoid arthritis, psoriasis, inflammatory bowel disease, cardiovascular disorders, neurodegenerative diseases, and metabolic syndromes [3,4].

Inflammation is broadly categorized into acute and chronic phases. Acute inflammation is characterized by increased vascular permeability, leukocyte infiltration, and edema formation, whereas chronic inflammation involves prolonged immune activation, tissue destruction, and fibrosis [5]. Key mediators such as TNF- α , interleukin-1 β (IL-1 β), interleukin-6 (IL-6), reactive oxygen species (ROS), and prostaglandin E2 (PGE2) play pivotal roles in amplifying inflammatory responses [6]. Conversely, anti-inflammatory cytokines such as interleukin-10 (IL-10) regulate and resolve inflammation. An imbalance between pro- and anti-inflammatory mediators leads to disease progression. Non-steroidal anti-inflammatory drugs

(NSAIDs) and corticosteroids are widely used to manage inflammation and associated pain. NSAIDs exert their effects by inhibiting cyclooxygenase (COX-1 and COX-2) enzymes, thereby reducing prostaglandin synthesis [7]. However, prolonged use of NSAIDs is associated with gastrointestinal ulceration, cardiovascular complications, renal impairment, hepatotoxicity, and increased risk of bleeding [8,9]. Regulatory agencies such as the US Food and Drug Administration have mandated safety warnings regarding cardiovascular and gastrointestinal risks associated with NSAID use [10]. These adverse effects necessitate the search for safer alternatives, particularly from natural sources.

Herbal medicine has gained global attention as a complementary and alternative therapeutic approach. Medicinal plants are rich sources of bioactive phytoconstituents such as flavonoids, phenolic compounds, alkaloids, tannins, and terpenoids, many of which possess anti-inflammatory, antioxidant, and immunomodulatory properties [11,12]. Polyherbal formulations, based on the principle of synergism, are widely practiced in traditional systems such as Ayurveda, Unani, and Siddha medicine. Synergistic interactions among phytochemicals may enhance therapeutic efficacy and reduce toxicity [13,14].

Cucumis sativus L. (family: Cucurbitaceae), commonly known as cucumber, is widely cultivated and traditionally used for its

cooling, anti-inflammatory, antioxidant, and anti-diabetic properties [15]. It contains flavonoids, triterpenes, vitamins (A, C, B6), potassium, magnesium, and phenolic compounds that contribute to its pharmacological potential [16]. Despite its traditional use, limited scientific evidence exists regarding the anti-inflammatory and immunomodulatory effects of *Cucumis sativus* peel. Immunomodulation represents a promising therapeutic strategy in inflammatory disorders. Targeting pro-inflammatory cytokines such as TNF- α while enhancing anti-inflammatory mediators like IL-10 has been shown to regulate inflammatory cascades effectively [17]. Therefore, the present study was undertaken to evaluate the anti-inflammatory and immunomodulatory effects of the ethanolic extract of *Cucumis sativus* peel (EECS) in carrageenan-induced experimental models. The study further aimed to assess its effects on serum TNF- α and IL-10 levels following single and repeated administration to provide scientific validation for its therapeutic potential.

Materials and Methods

Collection and authentication of the plant material:

The fresh three plant materials (*Citrulus lanatus* (watermelon) seeds, *Cucumis sativus* (cucumber) peel, *Psidium guajava* (guava) leaves) were collected from the local market of Sangareddy district, Telangana state, India. The individual plant parts were washed thoroughly under running tap water and shade dried at room temperature for 5 days. These samples were powdered in laboratory blender and were kept in airtight bottles until further use. The collected samples were authenticated morphologically by Dr. E. Narasimha Murthy, Department of botany, Osmania University, Hyderabad, Telangana state, India.

Preparation of polyherbal extract

Each one gram of extract contains an amount of *Cucumis sativus* (peel). 100g of the dried powder of each plant was taken and the extraction of these plant materials were carried out using soxhlet assembly with ethanol for about 36 h. The ethanolic extract was cooled and filtered. The filtrate was concentrated by using a rotary evaporator under reduced pressure till the concentrated mass was obtained. The yield of crude extract was stored in an air-tight desiccator's and used for further analysis.

Preliminary Phytochemical Analysis: The extract was tested for different phytoconstituents like alkaloids, glycosides, saponins, tannins, terpenoids, phenolic compounds, protein, carbohydrates using standard procedures.

Experimental design: The effect of a single dose of extract on carrageenan-induced paw oedema was studied in the first series of experiments. Animals were divided in five groups (n=6)

Group 1: Normal control received only saline.

Group 2: Diseased control (CGN)

Group 3: Standard receiving Diclofenac sodium 25mg/kg

Group 4: EECS 200mg/kg

Group 5: EECS 400mg/kg

Paw volume was measured prior to carrageenan injection and at the 2nd, 3rd, 4th and 24th hour after in order to determine the difference in the paw volume. The effect of repeated doses on carrageenan-induced paw oedema was studied in the second series of experiments. The experimental animals, the control and

positive control groups were treated as previously described in the first series of experiments but the treatment lasted 14 days and carrageenan oedema was induced on day 15. Paw volume was measured as described in the first series of experiments.

Drug administration

Diclofenac (25mg/kg), the EECS (dissolved in 0.1% CMC) were administered at a dose of 200 and 400mg/kg oral. Rats in the normal group and diseased group administered solely with an equal amount of normal saline. All drugs were prepared freshly every day. Doses were given according to the respective rat weights. Anti-inflammatory activity was evaluated using the carrageenan-induced rat paw edema model by injecting 100 μ L of 1% L-carrageenan into the right hind paw, and paw volume was measured at 0, 2, 3, 4, and 24 hours using a digital plethysmometer. Total phenolic content was determined by the Folin-Ciocalteu method and expressed as gallic acid equivalents (GAE/g), with absorbance measured at 725 nm. Total flavonoid content was estimated by the aluminum chloride colorimetric method and expressed as quercetin equivalents (QE/g), with absorbance recorded at 415 nm. In vitro anti-inflammatory activity was assessed by the HRBC membrane stabilization assay using hypotonicity-induced hemolysis, where percentage inhibition of hemolysis was calculated spectrophotometrically at 540 nm using diclofenac as standard. The egg albumin denaturation assay was performed to evaluate inhibition of protein denaturation, with absorbance measured at 660 nm and percentage inhibition calculated. Serum levels of TNF- α and IL-10 were quantified using ELISA and expressed in pg/mL.

Results and Discussion

Preliminary phytochemical analysis of polyherbal extracts

The polyherbal extract (PHE) were tested for different phytoconstituents like alkaloids, glycosides, saponins, tannins, terpenoids, reducing sugars, phenolic compounds, flavanoids, protein, carbohydrates and volatile oils using standard procedures and the results are given in following table.

Total Phenolic content: The total phenolic content of the ethanolic extract was determined by Folin-Ciocalteu reagent and expressed as Gallic acid equivalent per gram (GAE/g). The results have shown that the ethanolic extract was found to be high amount of phenols (161.05 $\pm$ 1.23 mg GAE/g). The phenolic compound is regarded as most powerful antioxidant.

Table 1: Preliminary phytochemical analysis of extracts

S. No	Phytochemical Tests	EECS
1	Alkaloids	+
2	Flavanoids	+
3	Terpenoids	+
4	Tannins	+
5	Saponins	+
6	Phenols	+

Table 2: Calibration of gallic acid for TPC

Concentration (μ g/ml)	Absorbance
100	0.124
200	0.234
300	0.442
400	0.663
500	0.886
600	0.914
1mg EECS	0.221

Table 3: Calibration of Quercetin for TFC

Concentration($\mu\text{g/ml}$)	Absorbance
100	0.703
200	1.141
300	1.176
400	1.591
500	1.662
600	1.929
1mg EECS	0.842

Table 4: Quantification of Flavonoids & Phenols

EECS	Standard	Mg/g dry mass
Phenolic (mg GAE /g)	Gallic acid	161.05 $\pm$ 1.23
Flavonoid (mg QE/g)	Quercetin	124.26 $\pm$ 2.12

Table 5. Effect of PHE and Diclofenac on hypotonicity induced hemolysis of HRBC membrane

Conc ($\mu\text{g/ml}$)	EECS Abs	Diclofenac Abs	% Prevention of RBC Haemolysis	
			EECS	Diclofenac
Control	1.038 $\pm$ 0.9	1.038	00	00
100	0.98 $\pm$ 0.12	0.90 $\pm$ 0.51	9.38 $\pm$ 0.20	17.09 $\pm$ 0.25
200	0.91 $\pm$ 0.21	0.82 $\pm$ 0.23	16.13 $\pm$ 0.34	24.80 $\pm$ 0.12
400	0.86 $\pm$ 0.23	0.76 $\pm$ 0.13	20.94 $\pm$ 0.32	30.58 $\pm$ 0.32
600	0.58 $\pm$ 0.14	0.56 $\pm$ 0.71	47.92 $\pm$ 0.25	49.85 $\pm$ 0.21
800	0.40 $\pm$ 0.32	0.34 $\pm$ 0.24	65.26 $\pm$ 0.21*	71.04 $\pm$ 0.21**
1000	0.32 $\pm$ 0.21	0.22 $\pm$ 0.12	72.97 $\pm$ 0.32*	82.60 $\pm$ 0.12**

Diclofenac was used as standard; Values are expressed as mean $\pm$ SD (n=6);

*p<0.05, **p<0.01 as compared with corresponding values of control values (oneway ANOVA followed by dunnett's test)

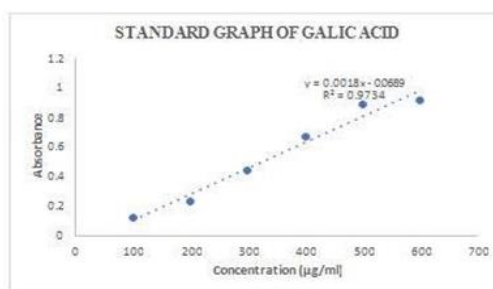
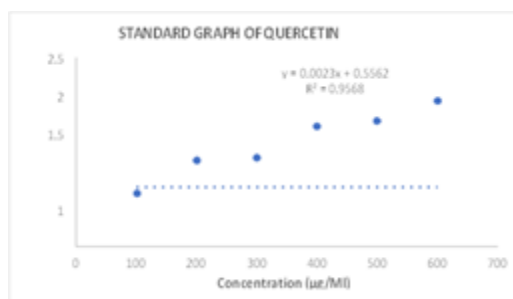
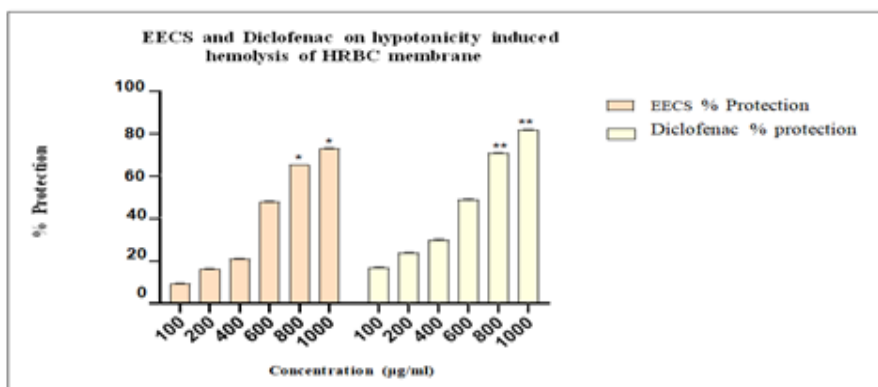
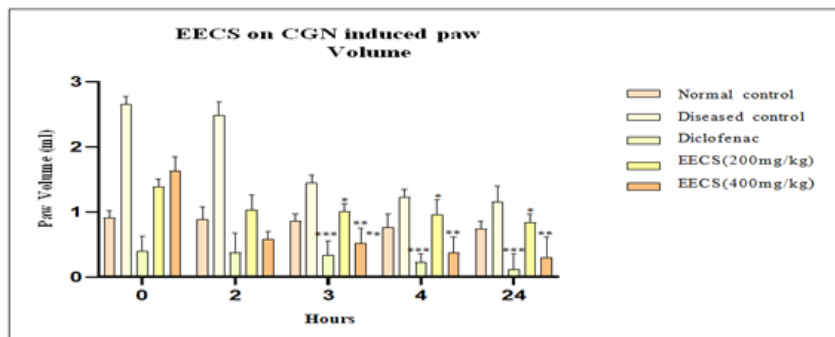
**Fig 1:** Total phenolic content of standard gallic acid calibration curve**Fig 2:** Total flavonoid content of standard Quercetin calibration curve**Fig 3:** Effect of extract and diclofenac on hypotonicity induced hemolysis of HRBC membrane

Table 6: Effect of EECS and Diclofenac on CGN induced paw volume

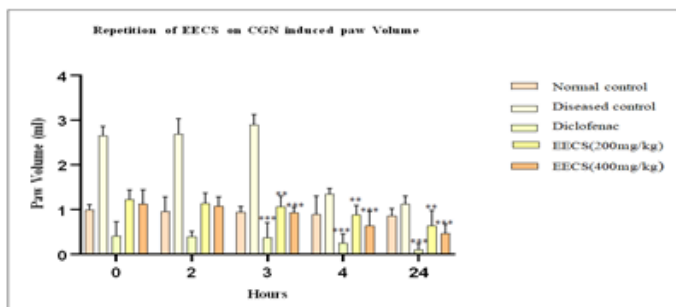
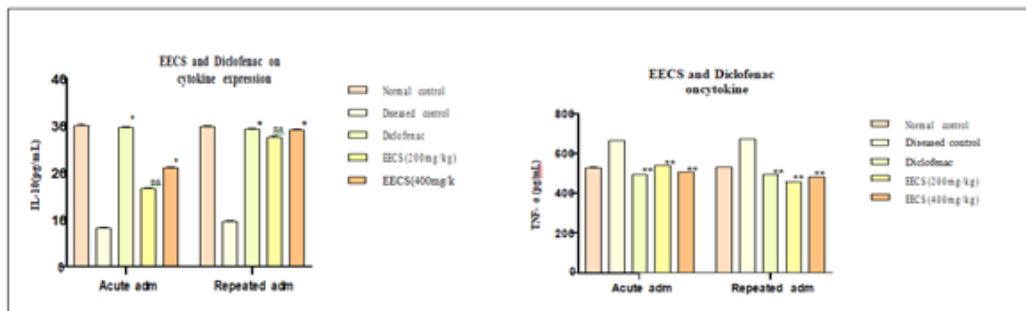
Groups	Time (hrs)				
	0	2	3	4	24
Normal	0.91±0.11	0.88±0.2	0.86±0.11	0.76±0.21	0.74±0.12
Disease control	2.65±0.12	2.48±0.21	1.45±0.12	1.23±0.12	1.15±0.24
Diclofenac	0.40±0.22	0.37±0.31	0.33±0.22***	0.23±0.13***	0.12±0.23***
EECS (200mg/kg)	1.39±0.12	1.03±0.23	1.00±0.13*	0.96±0.23*	0.84±0.13*
EECS (400mg/kg)	1.63±0.22	0.58±0.12	0.52±0.23**	0.37±0.24**	0.30±0.31**

*p<0.05, **p<0.01, ***p<0.001 (one way ANOVA accompanied by dunnett's test)

**Fig 4:** Results paw volume**Table 7:** Effect of repeated administration of PHE and Diclofenac on Carrageenan induced paw Volume

Groups	Time (hrs)				
	0	2	3	4	24
Normal	0.99±0.12	0.96±0.32	0.94±0.13	0.89±0.41	0.86±0.16
Disease	2.65±0.21	2.69±0.34	2.89±0.23	1.34±0.13	1.12±0.18
Diclofenac	0.41±0.32	0.39±0.12	0.37±0.34***	0.25±0.21***	0.10±0.14***
EECS (200mg/kg)	1.23±0.21	1.14±0.23	1.06±0.24**	0.88±0.21**	0.64±0.34**
EECS (400mg/kg)	1.12±0.32	1.07±0.21	0.93±0.12***	0.64±0.32***	0.46±0.21***

*p<0.05, **p<0.01, ***p<0.001 (one way ANOVA followed by dunnett's test)

**Fig 5:** Effect of repeated administration of EECS and Diclofenac on Carrageenan induced paw Volume**Fig 6:** Effect of PHE and Diclofenac on cytokines expression (pg/mL)

Statistical Analysis

The results are expressed as mean ± SEM using Graph Pad Prism. We used one-way analysis of variance (ANOVA) followed by multiple comparisons Dunnett's test. The statistical method applied in each analysis was described in

each figure. Results were considered to be significant when p-values were less than 0.05 (p < 0.05).

Discussion:

This study demonstrates that the ethanolic extract of *Cucumis sativus* peel (EECS) possesses significant anti-inflammatory

activity in the carrageenan-induced rat paw edema model. EECS is rich in phenolics and flavonoids (161.05 ± 1.23 mg GAE/g and 124.26 ± 2.12 mg QE/g), contributing to its antioxidant potential. The extract showed concentration-dependent membrane stabilization and inhibition of protein denaturation, indicating additional anti-inflammatory mechanisms. EECS significantly reduced paw edema in both phases of inflammation, likely via cyclooxygenase inhibition and prostaglandin suppression. It also decreased TNF- α and increased IL-10 levels, confirming its immunomodulatory and NSAID-like effects.

Conclusion

The findings of the present study showed that EECS possesses an anti-inflammatory effect in a carrageenan-induced model of inflammation after single and repeated administration. This action may be useful in the treatment of some inflammatory conditions. PHE showed an immunomodulatory effect that it decreased the levels of the pro-inflammatory cytokine TNF- α after single and repeated administration. An increased level of the anti-inflammatory cytokine IL-10 was observed more significantly after a repeated administration of EECS.

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